Actin gels
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Solutions and gels of the filamentous protein F-actin have not only been the subject of numerous recent biophysical studies, but have also proven to be quite fruitful model systems for fundamental polymer science. They are particularly good models of 'semiflexible' polymers, because they have an enormous ratio of persistence length to molecular diameter with characteristic mesh sizes of the order of one micron, which has permitted both a direct optical visualization of these networks and a number of recent experimental techniques for local viscoelastic characterization.

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Introduction and background
Biopolymers are becoming more commonly used to design biomaterials and as model polymers for fundamental polymer studies, for example for testing theories of polymer dynamics [1-9,10*,11**]. In part the attraction of these polymers is due to their enormous contour lengths and stiffness as compared with synthetic polymers. A particularly interesting and common example of this can be found in nearly all eukaryotic, or plant and animal cells. The outer rim of these cells contains a complex and highly dynamic composite of filamentous actin (F-actin), together with a range of accessory proteins for initiating and terminating polymerization introducing crosslinks, and forming lateral arrays of these filaments [12]. An electron micrograph of such an actin network near the cell periphery is shown in Figure 1. Reconstituted solutions of F-actin have been widely studied in vitro. These filaments can be up to several tens of microns in length, and their persistence lengths \( \lambda_p \) (the length over which the orientation of a macromolecule is preserved) have been measured to be of the order of 10 microns. These features, together with the availability of fluorescent labels for actin, have enabled the direct visualization of single polymer strands within fully hydrated three-dimensional networks [4,13]. The most interesting physical properties of actin gels are those that seem to be intimately related to their biological function in providing viscoelasticity to the cytoplasm. Actin polymers have a very high gelation efficiency and form viscoelastic gels at volume fractions below 0.1% (1 mg/ml). In contrast to most synthetic polymer networks, actin gels have the unusual property of strain hardening which is shared by some other cytoskeletal filaments. Analysis and modeling of these features have led to new theoretical insights into the way in which semiflexible polymers interact with each other in entangled and crosslinked networks.

Figure 1

An electron micrograph of macrophage cytoskeleton (courtesy of John Hartwig).

Actin filaments as biopolymers
F-actin is a double-stranded helical filament comprised of G-actin monomers, each of which is itself a globular protein of molecular weight 42,000. In buffers of physiological ionic strength (150 mM), globular actin subunits assemble by a polymerization reaction resembling the nucleated condensation of bifunctional monomers to form helical filaments of 6–10 nm diameter. Recent microscopic measurements of single filament flexibility in various geometries have revealed many features of filament dynamics and suggest that there is still much to learn about the structure and motions of actin filaments. Measurements of bending elasticity, derived from analysis of the thermal motions of F-actin, yield a variety of values ranging from 0.3–15 microns or more for the persistence length [6,7,13–15]. A biochemical study has shown the
significant effects of such ligands as divalent cations on actin structure [16], but the measured dependence of flexibility on ionic conditions is too small to explain the observed discrepancy among various studies [6,17**]. In contrast, inferences about F-actin flexibility derived from recent viscoelastic measurements have suggested that the persistence lengths are of the order of 0.5 microns [18]. Crosslinking of actin networks by alpha-actinin can transform isotropic entangled networks into heterogeneous networks of microgels, whose macroscopic viscoelasticity is a complex of contributions from both dense and loose meshworks [19*].

In part the calculated stiffness based on thermal fluctuations depends on modeling the actin filament as a uniform cylinder with a scale-independent bending modulus that is not coupled to such motions as torsion or internal rearrangement of the monomer–monomer contacts. These assumptions are consistent with some measurements [20**], but are called into question by a normal mode analysis of the F-actin structure based on X-ray data [21,22]. This work shows a multiplicity of possible motions including longitudinal pressure waves, torsional waves, bending, and motions such as a ‘groove-swinging’ motion of the two long-pitch helices, and axial slipping of the two strands unique to F-actin. These motions, some of which are experimentally observed [23**,24–27] along with probable packing defects within filaments [28,29*] may account for the usually dynamic state of F-actin and the difficulty in reaching a simple consensus regarding a view on filament stiffness. Two recent studies of filament torsion, based on twisting motions of single filaments observed by microscopy [17**] or by micromanipulation [20**] gave values of torsional stiffness much higher than earlier measurements of torsion by polarization of various covalently linked probes.

One important finding of some of this work is that cooperative conformational changes can be propagated at long distances along the actin filament by only subtle alterations in monomer–monomer packing [25,27,30]. This effect has many implications for structural reorganization of F-actin in myofibrils and other cellular structures. Additional interesting results include changes in the partial specific volume of the actin subunit after polymerization [31] and osmotic effects [32] on actin filament structure and its tendency to undergo bundling transitions [33*].

**F-actin solutions as models for polymer science**

In vitro solutions of F-actin exhibit a polydisperse distribution of lengths ranging from less than 1 μm to about 70 μm, with a mean length of ~20 μm [34]. At concentrations between a few micromolars per milliliter and 2 mg/ml these filaments form viscoelastic solutions [13]. In this concentration range, the mesh size ξ, or typical spacing between neighboring filaments, is in the range of 0.2 μm to a few micrometers, as judged by electron micrographs, simple geometric arguments, observation of the transverse motions of single labeled filaments [4,13], and diffusion of small beads [35]. An image obtained by fluorescence microscopy is shown in Figure 2. Above ~2 mg/ml, F-actin forms liquid crystalline, anisotropic phases [13,34,36–38]. In vivo, the actin cortex consists of an entangled meshwork of shorter actin filaments (of the order of 1 μm in length) at a concentration near 10 mg/ml, together with a pool of monomeric G-actin (at levels of a few milligrams per milliliter, which is kept monomeric by sequestering proteins [39]) and locally formed bundles of filaments. The isotropic network of actin filaments in a typical cell is characterized by a mesh size of approximately 100–200 nm (see Fig. 1).

As with other polymer solutions, the response of F-actin solutions to shear deformation can be measured by a variety of rheological experiments. Following the rapid application of a finite shear strain, F-actin solutions show an initially high stress that relaxes slowly and apparently without limit [40]. The response of this solution to an oscillatory shear strain exhibits a complex modulus (the ratio of stress to strain) which is characterized by an in-phase or real part (known as the storage modulus) and out-of-phase or imaginary part (the loss modulus). Over a broad frequency range (0.1–10 Hz) these solutions appear to be almost purely elastic [41]. The storage modulus G' depends very weakly on frequency and is larger than the loss modulus G″, resembling qualitatively the features of a highly entangled or crosslinked rubber. Despite some
similarity of the rheological properties of actin networks with conventional polymer gels and rubbers, classical rubber elasticity cannot account for either the large shear moduli required of the cell cortex in vivo or for the moduli measured for F-actin in vitro. F-actin networks also have a very small range of linear viscoelastic response, and an apparently small yield strain: for strains larger than about 10%, the response ceases to be linear or the networks rupture [41]. This yield strain is also seen to decrease with increasing concentration of the network. For comparison, conventional gels and rubbers exhibit a larger regime of linear elasticity, and tend to exhibit a decrease in G’ rather than an increase with moderately increasing strain. A further characteristic of F-actin is the concentration dependence of its elastic modulus. It has been shown that the shear elastic modulus of =1 mg/ml of F-actin increases with concentration, c, as $c^{x}$, where x is slightly larger than two [41]. Although this exponent is comparable with that predicted by classical rubber elasticity of flexible polymers, the observed values of the shear moduli in actin solutions are many times larger than such theories predict. Such a disagreement, however, is not surprising, because the microscopic structure of the network, clearly does not exhibit randomly-coiled chains on the scale of the mesh size of the network, as can be seen in fluorescently labeled actin filaments in a network of unlabeled filaments [13]. Between contact points within the mesh the actin filaments exhibit small, transverse thermal undulations about an otherwise straight shape due to the intrinsic stiffness of F-actin. Common random coiled configurations are not observed.

**Theory and modeling of actin gels**

Perhaps the most fundamental question concerning the origin of the viscoelastic response of actin solutions and gels is whether and to what extent this response is entropic in origin, as is the case of conventional polymer systems over a wide range of frequencies [42]. Due to the significant rigidity of actin filaments, there may be an important, even dominant contribution, due to the direct bending of filaments at a microscopic level (an enthalpic effect) when a network of such filaments is strained. Models based on both aspects, entropic and enthalpic, have been recently proposed [43,44-46*].

MacKintosh et al. [43] and Isambert and Maggs [44*] have proposed rather different models that can be regarded as entropic mechanisms. In the first of these [43], the elastic restoring force is assumed to be dominated by the resistance of single, fluctuating semiflexible filaments to an applied tension. The force-extension relationship, or spring constant due to transverse thermal fluctuations is much stronger than that of a flexible, random coil polymer [43,47]. The macroscopic shear modulus is calculated in this model, assuming a densely crosslinked network subject to affine shear. These results can be extended to uncrosslinked solutions, provided that the filaments are long enough to form entanglements that can support stress. The typical distance between these is defined to be the entanglement length, l_e. In [43], it is assumed that this length is proportional to the typical distance between steric constraints of a given filament due to its neighbors [48]. This results in an enhancement of the shear modulus by as much as a factor of 100 to 1000 over classical rubber elasticity for actin gels in the range of a few milligrams per milliliter, where the ratio $l_e / \kappa$ is large. However, this model assumes either the presence of crosslinks, or entanglements that support longitudinal stresses in the filaments. In contrast, Isambert and Maggs [44*] have shown that in the absence of such entanglements, the modulus is expected to be much smaller. Thus, the question of entanglement is a fundamental one for the origin of the apparent high shear moduli of actin solutions. The model of [44*] is based on the loss of configurational entropy under shear of a solution of semiflexible rods that experience only lateral constraints (entanglements) in an effective tube due to their neighbors [44*,49].

Satcher and Dewey [45*] and Kroy and Frey [46*] have recently suggested models that include direct contributions to the shear modulus from the bending of semiflexible filaments under network deformation. Satcher and Dewey began with an apparent analogy between the microscopic appearance of actin filaments, as observed in the electron microscope, and the fibers comprising materials such as paper and cotton wool. They calculated the compression modulus for a regular, orthogonal network of stiff rods, and assumed that the same form existed for the shear modulus of a gel. In a similar way, Kroy and Frey calculated the response of a rigidly-anchored semiflexible filament, including both direct bending and thermal fluctuation contributions to the force-extension relationship [43,47]. In applying either model to semiflexible solutions and gels, determining the role of direct filament bending is subtle. Although the specific geometry of the network may not significantly affect the enthalpic contribution of filament bends to the compression modulus [45*], in a more disordered network, that includes filaments crosslinked diagonally, it is not clear if significant bending of filaments really occurs. In the case of pure, affine shear (in which the strain is uniform) no bending of filaments occurs unless rigid, angle-preserving crosslinks are assumed to exist between the filaments [45*], or unless individual chains are effectively anchored at points where the orientation is fixed [46*]. Thus stretching and compression of filaments, which are included in the model of Kroy and Frey, can also significantly contribute to the restoring force of a sheared network. The approach of Kroy and Frey may provide a way of more quantitatively characterizing the roles of filament bending and stretching. The relative contributions of these depends on the degree to which the shear is nonaffine, as may occur either for an inhomogeneous network, or to the small but finite volume fraction of filaments.
Micromechanical manipulation, microscopy, and local viscoelastic characterizations

A major advance in rheological characterization has been the recent development of a variety of new microscopic rheological methods \cite{6,10,11}. Efforts in microscopic viscoelastic characterization actually go back to studies of gelatin elasticity using magnetic particles in the 1920s \cite{50}. Later modifications enabled the use of this technique in living cells \cite{51}, and in studies of spatial heterogeneities in mucous \cite{52}. More recent developments have been made possible by new experimental micromechanical techniques for local force generation, high resolution detection, and manipulation of small particles in a range of soft materials. These devices include optical tweezers \cite{53-55} and magnetic bead rheometers \cite{56}. In polymeric materials in particular, these tools have led to the development of so-called microrheology, or local viscoelastic characterizations. There are several potential advantages of microrheological techniques, especially as applied to polymer and biopolymer systems. First, these methods may permit the study of polymer networks at a microscopic scale (e.g. approaching the characteristic mesh size of the networks), thus making possible the direct characterization of heterogeneities within polymer networks. Perhaps even more important for biological applications, however, microrheology may permit the characterization of small samples, such as individual cells.

These microrheology techniques fall into two classes: those involving active manipulation of probe particles within the sample \cite{8,10,11}, and those employing passive observation of thermal fluctuations of probe particles within the sample \cite{57,58}. In either case, the probes used are typically relatively inert spherical beads of approximately one micron in diameter.

Ziemann et al. \cite{8}, Schmidt et al. \cite{11}, and Amblard et al. \cite{10} have used magnetic field gradients for direct manipulation of micron size magnetic beads to measure the viscoelastic response of actin gels. These groups measured the particle displacement by video microscopy. In \cite{8}, for instance, the authors reported measurements of both the storage modulus $G'(\omega)$ and loss modulus $G''(\omega)$ as a function of frequency, while Schmidt et al. \cite{11} described a technique to map the strain field by analysis of the motions of nonmagnetic beads in the vicinity of a magnetic probe bead (this is illustrated in Fig. 3). The forces applied are accurately determined by calibrating motion in viscous fluids, but knowledge of the strain is complicated by both geometric considerations and the finite mesh size or homogeneity of the network. Initial studies assumed application of point forces within a continuous, incompressible elastic medium \cite{8}. In general, values of elastic moduli determined from these studies are somewhat lower than those derived from bulk measurements and at present limited to studies of very low actin concentrations (0.075–0.3 mg/ml), where the degree of chain entanglement may not be representative of the situation at higher concentrations, where elastic moduli are higher and the mechanical loss lower \cite{59,60}. It has also been argued that the resistance to motion of small beads may be significantly different from that in bulk measurements owing to the finite number of actin strands in contact with the deforming elements, and that therefore parameters such as shear moduli may be difficult to deduce from such measurements \cite{10}. This may be particularly important at lower concentrations, where the mesh size and bead size become comparable—making the treatment of the medium as continuous a dubious approximation. In \cite{10}, the authors argued for a somewhat different physical origin of the drag force on a displaced sphere. In particular, in dilute solutions, they reported subdiffusive motion (in which the mean-square particle displacement increases with time less rapidly than expected for simple diffusion) over distances from the limit of optical resolution up to a few microns.

In contrast to the above micromanipulation techniques, it has been shown that imaging of thermal fluctuations of embedded micron-size beads can be used to measure the viscoelastic parameters of a polymer solution or gel \cite{57,58}. Alternatively, multiple scattering of light by embedded particles can also be used to measure viscoelastic parameters in soft materials \cite{61,62}. In \cite{57}, for example, a high-resolution technique is described for measuring the power spectrum of the spatial fluctuations of dielectric particles over a frequency range from 0.1 Hz to over 10 KHz. Not only can this technique be used to measure particle displacement over much shorter time
scales than can be done by video tracking, but it is also sensitive to much smaller particle displacements. As a result the shear modulus can be measured over a wide frequency range in soft materials (e.g. for shear moduli less than 10 Pa). As for the active methods described in [8,10*,11**], extracting the shear moduli from analysis of the thermal fluctuations in [57*,58*,61] assumes that a continuum viscoelastic media exists. This assumption may only be valid for probe particles of radii substantially larger than the characteristic mesh-size of the networks studied. Furthermore, a somewhat more subtle effect limits the measurement of shear moduli in polymer solutions at low frequencies. The viscoelastic response of polymer solutions involves contributions from both solvent and polymer. Theoretical treatments of this aspect of solution behavior include so-called two-fluid models, in which a phenomenological, viscous coupling is assumed to couple the network to the solvent [63,64]. At sufficiently high frequencies, this viscous coupling effectively forces the two components to move as a single fluid (i.e. there is little or no 'draining' of the network). This means that a treatment of the solution as a continuum, incompressible viscoelastic medium is valid [57*]. However, at low frequencies, the two components begin to decouple, leading ultimately to an elastic medium with a Poisson ratio less than 1/3 in the limit of very low frequencies [11**].

**Application to biology**

The biological relevance of understanding actin's viscoelasticity relates to its influence on cell mechanics and motility and to possible modes of the mechanotransduction of signals initiated at the cell surface to the nucleus or other intracellular sites [65,66*]. For example, direct measurements of cell stiffness show that local stiffness increases in direct proportion to the amount of deforming stress [66*], a result in stark contrast to the elastic response of rubber-like gels and even stiffer gels such as starch, which exhibit a linear (stress-independent) elastic modulus at equivalent strains. One interpretation of such data is that cell mechanics is governed by a tight coupling between semiflexible elements (actin-myosin) and incompressible struts (perhaps microtubules) and an analogy has been made with the architectural model of tensegrity in which such elements are combined to make free standing structures [66*]. An alternative view derived from rheological studies of F-actin [43,67], as well as other semiflexible biopolymers like fibrin [68] or neurofilaments [69], is that networks of these polymers are intrinsically strain-hardening, and therefore the stress-dependent cell stiffness is consistent with passive mechanical resistance. The magnitude of the actin cytoskeleton's elastic modulus also helps to define the extent to which activation of myosin motors will induce flow or deformation of the cell cortex, and the extent to which the cytoskeleton can be deformed by the low fluid shear stresses that activate gene expression in cells such as endothelial cells [45*,70]. The magnitude of the individual filament bending modulus, which may differ for different actin isotypes [59], also plays an important role in governing the amount of force that can be generated by thermal ratcheting mechanisms [71**] proposed to propel bacteria and viruses [72,73] through the cytoplasm and the extension of lamellae [74].

**Discussion and future prospects**

Biopolymer systems such as actin solutions should continue to provide excellent model systems for a number of future fundamental studies of polymer structure and dynamics. A number of observations have been made that suggest possible avenues of future study. First of all, these systems pose an interesting challenge to polymer theory: namely, to develop a satisfactory model for solutions of semiflexible polymers. Biopolymer systems such as actin show that an alternative to classical rubber elasticity of flexible chains is needed for semiflexible solutions and gels. The range over which we expect the semiflexible nature to be apparent for gels and solutions can be characterized in terms of the aspect ratio \( l_p/a \) of persistence length to diameter \( a \). On the one hand, the concentration below which the chains can be treated as flexible is determined by the condition that the mesh size, \( \xi = (ac)^{1/2} \), becomes comparable to the persistence length. Flexible behavior is therefore expected for low concentrations: \( ac < l_p^2 \), provided that molecular weight is sufficiently high. On the other hand, a liquid crystalline phase is expected at higher concentrations [75]: \( c > (a l_p)^{-1} \). Thus the range of concentrations for which semiflexible behavior can be expected is \( (a l_p)^{1/2} < \phi < (a l_p) \), where \( \phi = c a^3 \) is the volume fraction. While there are numerous synthetic examples of semiflexible polymers, the appearance of a broad semiflexible regime of elasticity depends on a large aspect ratio \( l_p/a \). Such is the case of almost all biopolymers, including one of the more flexible biopolymers, DNA, for which this ratio is still of the order of ten.

One of the more fundamental challenges for polymer theories of such solutions of semiflexible systems concerns the nature of entanglements in these systems. While models such as [48] have begun to characterize these entanglements, the analogy with entanglements of flexible chains remains an open question for future research. In particular, it is important to note that in semiflexible systems such as actin solutions, chains cannot form loops or knots on the scale of their typical separation \( \xi \). Therefore, the nature of molecular constraints between filaments must differ from entangled random coils.

Another intriguing possibility for future experimental studies in biopolymer systems, such as actin, is the prospect of basic studies of the nature of microscopic strain in solutions and gels. It has been recently suggested [76] that a significant reduction in the shear modulus of gels can result from fluctuations or deviations of strain from simple affine shear (characterized by uniform shear strain, in which the local strain is equal, everywhere, to the macroscopic strain). Because individual actin filaments can be fluorescently labeled for direct visualization by optical
microscopy, it should be possible to examine the nature of strain down to the scale of the mesh size under applied shear.

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:
• of special interest
•• of outstanding interest


The authors describe an apparatus for microrheological characterization using magnetic beads together with video tracking of particle motions. They report anomalous, subdiffusive bead motion in actin solutions.

12. The authors demonstrate a method of observing the shear field in the neighborhood of a single large magnetic bead, by video tracking of small non-magnetic beads in the field of view. This method can be used to determine the Poisson ratio of a polymer network at low frequencies, or possibly for observing inhomogeneities within a solution.


By attaching micron-sized beads to the side of an actin filament whose ends are immobilized, the torsional rigidity of the filament is analyzed from the rotation of the beads due to thermal motions of the filament. The result is a torsional rigidity of the order of 5 x 10-28 Nm² which is much larger than previous measurements from spin labels or polarized luminescence. Small chemical differences in the filaments (Ca2+ versus Mg2+ bound to the subunits) altered torsion much more than bending elasticity, suggesting that these modes of motion are structurally uncorrelated.


Elegant combination of optical trapping and micromanipulation apply both torsion and elongational stress to a single filament to determine rigidities and breaking forces. The torsional rigidity (8 x 10-28 Nm²) is very close to that measured by Yasuda, and consistent with a uniform cylinder with measured bending modulus. A surprisingly small twist (90 deg) of a filament with thousands of subunits greatly reduced the force needed to break the filament under elongational stress.


Quasielastic light scattering is used to investigate the effect of the tropomyosin/triton (Tn/Tn) complex on the bending stiffness of actin filaments. The bending stiffness is found to increase with binding of the Tn/Tn complex.
The authors determine the force&tension relation for rigidly anchored wormlike filaments. The compression modulus for this is calculated. The authors find the actin cortex is modeled by a regular scaffolding of crosslinked semiflexible actin filaments. A quantitative analysis of the transition from isotropic networks to bundled structures caused by molecular crowding and how the effects of end-capping proteins alter this transition by not only changing filament length, but by inducing cooperative reorganization of the filament.

The shear modulus arising from orientational entropy is calculated for wormlike chains, including both entropic elasticity and direct bending contributions. Implications of this for the plateau modulus of semiflexible polymer gels are discussed.

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A quantitative analysis of how the elasticity of actin networks, and the flexibility of single filaments can provide the directed motion required for protrusive of the cell periphery.


